Trop J Nat Prod Res, August 2023; 7(8):3652-3657 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Antioxidant and Hepatoprotective Activities of *Paris polyphylla* Sm. Rhizome Extract in Cyclophosphamide-Induced Hepatic Damaged Mice

An G.T. Nguyen[1], Hieu T. Tran[2], Chen V. Tran[3], Binh N.D. Pham[4], Hieu N. Nguyen[5], Hoa N. Nguyen[6], Khoa V. Vu[7]*

[1]*Faculty of Biology, College of Education, Vinh University, Nghe An Province, Vietnam*
[2]*Department of Chemistry, College of Education, Vinh University, Nghe An Province, Vietnam*
[3]*Faculty of Traditional Medicine, University of Medicine and Pharmacy at Ho Chi Minh City, Vietnam*
[4]*Van Lang High-tech Research and Development Institute, Van Lang University, Ho Chi Minh City, Vietnam*
[5]*Faculty of Pharmacy, PHENIKAA University, Hanoi, Vietnam*
[6]*Stroke Center, Nghean General Friendship Hospital, Nghe An Province, Vietnam*
[7]*Surgeon of Orthopedic and Trauma, Deputy Department of Orthopedic and Outpatient Treatment, Vietduc University Hospital, Ha Noi, Vietnam*

ARTICLE INFO

Article history:
Received 01 June 2023
Revised 22 June 2023
Accepted 27 June 2023
Published online 01 September 2023

ABSTRACT

Paris polyphylla Smith (Melanthiaceae) is a medicinal plant for treating inflammatory, cancer, and liver-related diseases. Information on *in vivo* hepatoprotective effects of *P. polyphylla* rhizome extract has not been reported in the literature. The purpose of this study is to determine the antioxidant and hepatoprotective effects of *P. polyphylla* rhizome extract in cyclophosphamide-induced hepatic damaged mice. The beneficial effects on the liver of P. polyphylla extract have been evaluated through biochemical indicators (AST, ALT). Antioxidant activity was assessed by measuring levels of hepatic malondialdehyde (MDA) and antioxidant glutathione (GSH). A histopathological examination was also performed. *P. polyphylla* extract had DPPH free radical scavenging and lipid peroxidation inhibitory activity with IC_{50} values of 389.15 ± 9.09 µg/mL, 164.06 ± 8.32 µg/mL, respectively. Simultaneously, ALT, AST, and MDA contents decreased, whereas that of liver GSH of mice increased when they received oral doses of 0.75 and 1.5 mg/g. Histopathological analysis of the liver revealed a significant hepatoprotective effect of *P. polyphylla* extract (1.5 mg/g) with evidence that no histopathological damage was found, there was a reduction in lipid changes, and the degree of necrosis in the cells was reduced. The findings in my control study are evidence of the hepatoprotective potential of *P. polyphylla* extract, indicating that it might be used to prevent and treat liver-related diseases.

Keywords: Antioxidant, Cyclophosphamide, Hepatoprotection, Lipid peroxidation, *Paris polyphylla* Sm

Introduction

The liver is the organ responsible for metabolism and detoxification in the body.[1] The liver is vulnerable to toxins such as drugs, chemicals, viruses, and attack by free radicals.[2] Excess free radicals can cause oxidative stress in the body, where the formation of free radicals is out of balance with antioxidant activity that affects cellular structure. This phenomenon is generated during cell activity, with the strong formation of reactive oxygen species (ROS).[3,4]
ROS is formed by endogenous factors, including oxygen free radicals such as superoxide anion (O_2-), hydrogen peroxide (H_2O_2), hydroxyl radical (HO•), and exogenous factors (e.g., viruses, smog, chemicals, etc).[3] In addition, the sudden increase in intracellular ROS levels can lead to significant damage to cell structures, membrane permeability disorders, protein denaturation, lipid oxidation, genetic changes in the cell nucleus, and even cell death.[4]

*Corresponding author. E-mail: drkhoavu@gmail.com
Tel: +84-97-2016688

Citation: Nguyen AGT, Tran HT, Tran CV, Pham BND, Nguyen HN, Nguyen HN, Vu KV. Antioxidant and Hepatoprotective Activities of *Paris polyphylla* Sm. Rhizome Extract in Cyclophosphamide-Induced Hepatic Damaged Mice. Trop J Nat Prod Res. 2023; 7(8):3652-3657 http://www.doi.org/10.26538/tjnpr/v7i8.13

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

The destruction of ROS has been implicated in carcinogenesis, cardiovascular disease (e.g., atherosclerosis, diabetes, ischemic heart disease), cirrhosis, neurodegeneration, and aging.[5,6] When the body has long-term liver damage, it can cause chronic liver diseases. To ensure that the body can avoid the bad effects of ROS, cells have equipped themselves with a protective system, which is antioxidants. These compounds have the ability to slow down, prevent or reverse the oxidation of compounds present in the body's cells.[7] The body's antioxidants can be obtained from outside or inside of the body. The endogenous antioxidant system includes proteins (ferritin, transferrin, and albumin) and antioxidant enzymes, including lipid peroxidation (LPO).[8] Meanwhile, the exogenous antioxidant system includes tocopherol, ascorbic acid, carotenoids, phenolic compounds, and saponins.[9]
In previous studies, the natural antioxidants found in plants with strong antioxidant and free radical scavenging properties are considered bioactive substances with health benefits.[10,11] In literature, many studies have reported the anti-inflammatory, antioxidant activities, and hepatoprotective effects of plant extracts.[12]
Paris polyphylla Sm. and other *Paris* species are rare medicinal plants in Vietnam and the world.[13–15] In Vietnamese traditional medicine, *P. polyphylla* has been used as an antidote to snake bites, anti-inflammatory, anti-tumor, etc. Previous studies reported that *P. polyphylla* contains phytochemicals with many beneficial biological effects on health, such as flavonoids, phenolics, steroids, terpenoids, saponins, etc.[16–18] Zhang *et al* isolated three more diosgenin saponins and demonstrated the roles of these compounds on the immune system of mice.[19] Wu *et al* isolated from the rhizomes fifteen triterpenoid saponins, including six oleanane-type compounds (paritrisides A–F).[20] Qin *et al* isolated and structurally elucidated six new spirostanol

saponins, including polyphyllosides A–F, from the stems and leaves.[21] Kang *et al* identified four steroid saponins compounds (parisyunnanosides G–I, parisyunnanoside J) that have inhibitory effects on human CCRF leukemia cells.[22] *P. polyphylla* has been used for the treatment of cancer (e.g., lung cancer, laryngeal cancer, carcinoma, etc.) or liver-related diseases.[23,24]

Cyclophosphamide is a drug widely used to treat types of lymphoma as Hodgkin, non-Hodgkin, lymphocytic and small lymphocytic, Burkitt, bone marrow cancer (multiple myeloma), and other cancers such as breast cancer and eye cancer (retinoblastoma).[25] When cyclophosphamide enters some organs of the body, it is biotransformed into aziridinium ion metabolism products with alkylation activity. They react and covalently bond with guanine residues on DNA to form cross-links between two DNA strands. As a result, this drug works by blocking DNA replication and transcription. The strongest effect of cyclophosphamide is the inhibition of the division of all proliferating cells, especially the liver. During hepatic metabolism, cyclophosphamide is converted to potentially toxic substances that increase lipid peroxidation in the liver.[24] Therefore, this study was conducted with the aim of evaluating the antioxidant capacity towards hepatoprotection of *P. polyphylla* rhizome extract in cyclophosphamide-induced hepatic damaged mice.

Materials and Methods

Plant material

P. polyphylla (fresh rhizomes) was collected from Phu Xai Lai Leng Mountain at an altitude of 2711m in Nghe An Province, Vietnam (19°11'52"N, 104°10'57"E) in August 2021. A voucher specimen (No. GiangAn-02010) was identified by Assoc. Prof. Thi Huong Le has been kept at the Faculty of Biology, College of Education, Vinh University, Nghe An Province, Vietnam.

Methods

Preparation of crude extract

After being harvested, the rhizomes were washed under tap freshwater, dried, and ground into midding particles. The rhizome powder was mixed with 80% ethanol, the ratio of material: solvent (1:10 g/mL). This mixture was soaked for three days. The obtained extract was recovered by a rotary evaporator at 45 ± 2°C to obtain a crude extract and stored at 4°C until use.

Chemicals and reagents

Ethanol (OPC Pharmaceutical Company, Vietnam); methanol (Xilong Scientific Co. Ltd, China); glutathione (GSH) (TaiYu Chemical & Pharmaceutical Co. Ltd, Taiwan); eosin Y (BDH, UK); hematoxylin, ascorbic acid, Trolox (Merck Co. Ltd, Germany); endoxan® (Symbol: CYP, contains 500mg anhydrous cyclophosphamide) (Baxter International, Baxter Oncology GmbH, Germany); silymarin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), thiobarbituric acid (TBA), 5,5′-dithiobis (2-nitrobenzoic acid) (Ellman) (Sigma Co. Ltd, USA); NaCl, KCl, EDTA-Phosphate (Chemsol Co. Ltc, Vietnam). Other reagents and solvents were provided by Sigma-Aldrich (USA) at analytical grade.

Experimental animals and housing conditions

The male BALB/c mice (7–8 weeks old, 25.72 ± 0.71 g) were provided by the Institute of Biotechnology, Vietnam Academy of Science and Technology. The animals were allowed to freely access food and fresh water for one week. Additionally, these animals were set to a light (12 h)/dark (12 h) cycle at 25ºC to adapt themselves to environmental conditions. Treatment of laboratory animals and experimental procedures strictly followed the rules in the "Guidelines for the Treatment of Animals in Research and Behavioral Teaching" and adhered to ethical practices in animal research.

Experimental design

The experimental procedure was carried out based on a previous report by Pham *et al*. (2016)[26] Experimental mice were placed in 2 groups: the normal CYP (-) group and the CYP (+) group that was hepatotoxic induced by Endoxan® injection (cyclophosphamide 150 mg/kg of body weight). Each group was divided into 4 lots. Each lot contained ten mice (n = 10): Lot 1 was a normal control and received only distilled water; lots 2 and 3 were administered with *P. polyphylla* rhizome extract at doses of 0.75 mg/g and 1.5 mg/g of body weight at 8–9 AM, respectively; lot 4 received dose of silymarin 0.1 mg/g of body weight at 8–9 AM. All administrations were given orally for 7 consecutive days. On day 8, the experimental animals were sacrificed, blood collected, and the liver was excised for the subsequent assays.

Antioxidant assay by DPPH inhibition

The crude extract was determined for antioxidant activity using DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging assay based on the reported protocol by Al-Rimawi *et al*. (2020).[27] The crude extract was dissolved in ethanol solution and prepared with a range of concentrations, i.e., 50, 100, 250, 500, 1000, and 2000 µg/mL. 0.5 mL of sample solution was then used for the reaction with 0.5 mL of 0.8 mM methanol solution of DPPH. Then, the mixture was incubated in the dark at 25 ºC for 30 min. Ascorbic acid was selected as a positive control. Absorbance was determined by spectrophotometer (Shimadzu model UV-1601, Japan) at 515 nm. Linear regression was utilized to calculate the IC_{50} value (µg/mL).

Lipid peroxidation inhibition capacity assay

Inhibitory capacity on lipid peroxidation was measured using the crude extract originating from *P. polyphylla* rhizomes according to the slightly modified method.[28] Mouse brain was separated and prepared in 5 mM phosphate buffer with a ratio of 1:4 (w/v) at 0–5 ℃ and 1.4 mL of 5 mM phosphate buffer to obtain brain homogenate. The reaction mixture contained 0.1 mL of test samples solution (concentration of 50, 100, 250, 500, 1000, and 2000 µg/mL), 0.5 mL of the brain homogenate was incubated at 37 °C for 15 min, the reaction was stopped by adding 1.0 mL of 10% trichloroacetic acid. The reacted mixture was centrifuged at 10000 rpm for 10 min at 25 °C; the obtained solution was reacted with 1.0 mL of 0.8% thiobarbituric acid for 15 min at 100 °C. After being cooled for thirty minutes, the absorbance was recorded at 532 nm by spectrophotometer (Shimadzu model UV-1601, Japan). Trolox was selected as a positive control.

Biochemical parameters examination assay

Biochemical tests on alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were performed according to the method by Devaraj *et al.* (2014).[29] Mouse blood was solidified at 25 ºC for 60 min, then centrifuged at 3000 rpm for 15 min at room temperature to obtain serum. Biochemical parameters of the serum were determined using the Semi-automatic Biochemistry Analyzer AU-680 (Beckman Coulter, Japan).

Antioxidative enzymes analysis

Antioxidative enzyme analysis was conducted using the method of Pham *et al*.[26] Mouse liver was separated and then homogenized in KCl (1.15%) at 13000 rpm for 1 min. The reaction mixture contained 2.0 mL of homogenized tissue, and 1.0 mL of Tris (pH 7.4) incubated at 37 °C for 60 minutes; the reaction was stopped with 1.0 mL of trichloroacetic acid. After centrifugation at 5 °C with 10000 rpm for 10 min, the supernatants were prepared for biochemical analyses, including the glutathione (GSH) and malondialdehyde (MDA) tests.

Determination of glutathione (GSH) level: the mixture comprised the reaction of 1.0 mL of supernatant, 0.2 mL of Ellman, and 0.8 mL of EDTA-phosphate were measured for absorbance after 3 min at 412 nm. The GSH content (nM/g) was determined using linear regression of standard GSH.

Determination of malondialdehyde (MDA) level: the mixture comprised 2.0 mL of supernatant reacting with 1.0 mL of thiobarbituric acid (0.8%) at 100 ℃ for fifteen minutes. This mixture was then measured for absorbance at 532 nm. Trolox was selected as a positive control. The MDA content (nM/g protein) was analyzed according to the linear regression with the standard MDA.

Free radical scavenging percentage DPPH (%) was calculated using the formula below (A, %):

A (%) = (Control absorbance − Sample absorbance)/Control absorbance) × 100

Histopathological studies
Histopathology was evaluated according to Lu *et al*[30] with slight improvement. The small lobes of the mice's liver were stored in a 10% formalin solution and dipped into paraffin, and subsequently fixed onto the slides. After the conventional steps, sections 2-3 µm thick were obtained and fixed onto the slides, and stained with hematoxylin-eosin. Finally, tissue lesions were observed under light microscopy (Leica, Germany).

Statistical analysis
After repeating three times for each experiment, the results are presented as mean values ± SEM. Student's t-test was used to test the statistical difference between means. One-way ANOVA analysis and Dunnett's test were used for multiple data comparisons. MDA (nM/g of protein) and GSH (nM/g) content were determined using TableCurve 2Dv4 software. The difference is statistically significant when $p<0.001$, $p<0.01$ or $p<0.05$.

Results and Discussion

Potential antioxidant and lipid peroxidation inhibition
The results of antioxidant activity and lipid peroxidation inhibition of *P. polyphylla* rhizome extract are presented in Table 1. The antioxidant results of *P. polyphylla* rhizome extract at the concentration of 2000 µg/mL showed the same effect as ascorbic acid with IC_{50} values of 380.15 µg/mL and 363.32 µg/mL, respectively. In addition, a concentration of 250 µg/mL of *P. polyphylla* rhizome extract showed inhibition of lipid peroxidation of over 50%. Specifically, the rhizome extracts of *P. polyphylla* and Trolox had IC_{50} values of 164.06 ± 8.32 µg/mL, 386.28 ± 8.53 µg/mL, respectively. These results showed that the inhibition of lipid peroxidation in the MDA test of *P. polyphylla* is 2.4 times as high as that of the control trolox. In other words, the inhibitory ability of the extract was stronger than that of the positive control trolox. The antioxidant activities of *P. polyphylla* may have been related to phenolic compounds and flavonoids.[31,32]
In the body, antioxidants help protect proteins, lipids, DNA and other molecules from damage by oxidizing agents. This result discovers a better antioxidant capacity than the study of Mayirnao *et al*. with an IC_{50} value of 1090.0 µg/mL.[33] These results showed that *P. polyphylla* rhizome extract has good activity in inhibiting lipid peroxidation.

Hepatoprotective effects of *P. polyphylla* rhizome extract
Changes in mice body weight on a CYP model
The results of the mice's body weight before the experiment and after 8 days of cyclophosphamide injection in both CYP (-) and CYP (+) groups are presented in Table 2. In the group that was not injected CYP (-), the body weight of mice in all 4 lots increased compared to before the experiment ($p<0.05$); however, between experimental lots was comparable ($p>0.05$). After the mice were injected with CYP (+), they showed signs of anorexia and fatigue. By day 8 of treatment, these mice lost weight compared to before the experiment ($p<0.001$) and the non-CYP (-) group ($p<0.05$). After the mice were given *P. polyphylla* rhizome extract at doses of 0.75 mg/g and 1.5 mg/g or using silymarin 0.1 mg/g, their body weight in the CYP (+) injection group (lot 3, 4, and silymarin) was higher than the pathological control lot but not statistically significant ($p>0.05$). Thus, the use of extract or silymarin has an effect on the weight of mice. However, since the experiment time is short, this difference is not distinctive.

Hepatoprotective effects of P. polyphylla rhizome extract
Enzymes ALT and AST are two indicators that reflect the extent of damage when the liver is cancerous, toxic, cirrhosis, or viral infection. Therefore, the degree of liver damage is assessed through the levels of AST and ALT in the serum. The content of MDA and GSH in the liver was also determined to evaluate the liver injury and hepatoprotective ability of *P. polyphylla* rhizome extract. Oxidative stress and lipid peroxidation occurring in the liver are reflected through changes in MDA and GSH content. These results are presented in Table 3.
The results of Table 3 revealed that AST and ALT enzyme levels in the non-CYP (-) group of the experimental lots had no statistically significant difference ($p>0.05$). However, after being injected with CYP(+), the levels of AST, ALT, and MDA in all 4 experimental groups increased, while the GSH content decreased significantly compared to the group without CYP (-) ($p<0.01$). Notably, both lots 3 and 4 witnessed a decrease in AST, ALT, and MDA contents; meanwhile, GSH content increased after 1 week of treatment compared to the pathological control lot ($p<0.05$). Furthermore, the AST, ALT, MDA, and GSH contents in lot 3 were similar to the positive control lot with 0.1 mg/g dose of silymarin ($p>0.05$). In addition, the AST and MDA contents of lot 4 were almost similar to those of the normal control lot without CYP injection ($p>0.05$).
The liver is the organ that metabolizes and eliminates toxins in the body. Therefore, excessive oxygen free radicals can contribute to different stages of liver fibrosis.[34] Cyclophosphamide is widely used for cancer treatment. However, when entering the liver, cyclophosphamide is converted into 4-hydroxycyclophosphamide and aldophosphamide, leading to the formation of cytotoxic substances and increased lipid peroxidation in the liver.[35] Therefore, cyclophosphamide is often used in experimental models of liver damage. Previous studies demonstrated that silymarin was an antioxidant and an anti-inflammatory, inhibiting the process of liver inflammation into cirrhosis leading to liver cancer[36] and was against cyclophosphamide-induced liver damage.[37,38]
The extent of liver damage was expressed by serum transaminase enzyme levels.[39] In this study, mice that had been injected with cyclophosphamide showed lethargy and did not eat. Compared with the control group, after 8 weeks, the serum ALT and AST levels of mice increased significantly ($p<0.05$). This result proved that the mice had liver damage after cyclophosphamide injection. Surprisingly, the serum ALT and AST levels of the mice after being administered with *P. polyphylla* rhizome extract (0.75 mg/g) decreased to the same level as the therapeutic dose of silymarin 0.1 mg/g.

Table 1: *In vitro* antioxidant activity and lipid peroxidation inhibition of *P. polyphylla* rhizome extract.

Concentration (µg/mL)	DPPH radical scavenging activity A (%)	Ascorbic acid (positive control) A (%)	Lipid peroxidation inhibition A (%)	Trolox (positive control) A (%)
2000	89.30 ± 0.30	94.89 ± 0.50	89.23 ± 0.31	77.7 ± 1.43
1000	68.40 ± 0.04	94.19 ± 1.42	87.25 ± 0.42	67.7 ± 1.45
500	51.40 ± 0.12	60.84 ± 1.32	78.24 ± 1.23	63.49 ± 0.32
250	47.70 ± 0.12	28.31 ± 0.32	59.78 ± 0.32	32.37 ± 0.42
100	36.10 ± 0.30	16.76 ± 0.21	38.02 ± 0.02	19.96 ± 0.21
50	28.40 ± 0.30	5.59 ± 0.02	27.25 ± 0.04	4.86 ± 0.02
IC_{50}	380.15 ± 9.09	363.32 ± 10.29	164.06 ± 8.32	386.28 ± 8.53

Table 2: Mice body weight change before and after the experiments

Group	Lot (n = 10)	Dosages (mg/g of body weight)	Mice's body weight (g)*		*p*-value**
			Before the experiment (a)	After the experiment (b)	
CYP (-)	Normal control (1)	Distilled water	24.42 ± 0.86	27,50 ± 1.28	$p_{a,b} < 0.05$
	Lot 1 (2)	0.75	25.42 ± 0.45	28.33 ± 0.56	$p_{1,2,3,4} > 0.05$
	Lot 2 (3)	1.5	25.40 ± 0.56	27.70 ± 0.76	$p_{2,6} < 0.05$
	Silymarin (4)	0.1	26.18 ± 0.85	28.45 ± 0.98	$p_{3,7} < 0.05$
CYP (+)	Pathological control (5)	Distilled water	25.25 ± 0.72	22.58 ± 0.62	$p_{4,8} < 0.05$
	Lot 3 (6)	0.75	25.17 ± 0.63	23.25 ± 0.43	$p_{a,b} < 0.001$
	Lot 4 (7)	1.5	26.40 ± 0.67	23.90 ± 0.81	$p_{5,6} > 0.05$
	Positive control: Silymarin (8)	0.1	27.50 ± 0.91	23.70 ± 1.03	$p_{5,7} > 0.05$
					$p_{5,8} > 0.05$

* Mean ± SEM, n = 3. ** The difference was statistically significant when $p < 0.001$, $p < 0.05$, and vice versa

Table 3: Liver enzymes and MDA, GSH contents of mice after cyclophosphamide-treated

Group	Lot (n = 10)	Hepatic enzymes*		MDA content* (nmol/g)	GSH content* (nmol/g)
		ALT (U/L)	AST (U/L)		
CYP (-) (a)	Normal control (1)	35.40 ± 1.19	168.81 ± 1.52	92.32 ± 5.44	6638.83 ± 392.87
	Lot 1 (2)	36.10 ± 0.38	166.30 ± 0.66	90.46 ± 6.32	7270.15 ± 297.63
	Lot 2 (3)	35.60 ± 0.58	167.50 ± 0.76	89.03 ± 5.94	7142.85 ± 168.18
	Silymarin (4)	35.90 ± 0.80	167.10 ± 0.80	91.46 ± 8.96	7815.68 ± 185.38
	p-value**	$p_{1,2,3,4} > 0.05$	$p_{1,2,3,4} > 0.05$	$p_{1,2,3,4} > 0.05$	$p_{1,2,3,4} > 0.05$
CYP (+) (b)	Pathological control (5)	285.41 ± 0.38	443.67 ± 0.79	150.30 ± 9.92	4311.81 ± 164.63
	Lot 3 (6)	97.30 ± 0.74	185.30 ± 0.71	98.90 ± 5.68	5891.18 ± 163.14
	Lot 4 (7)	46.20 ± 0.77	165.10 ± 1.20	88.28 ± 8.36	6669.63 ± 168.95
	Positive control: Silymarin (8)	96.00 ± 0.99	194.90 ± 0.75	86.53 ± 2.47	5838.61 ± 231.68
	p-value**	$p_{5,6;\ 5,7;\ 5,8} < 0.05$; $p_{6,8} > 0.05$; $p_{1,5;\ 2,6;\ 3,7;\ 4,8} < 0.01$	$p_{5,6;\ 5,7;\ 5,8} < 0.05$; $p_{6,8;\ 3,7} > 0.05$; $p_{1,5;\ 2,6;4;8} < 0.01$	$p_{5,6;\ 5,7;\ 5,8} < 0.05$; $p_{6,8;\ 3,7} > 0.05$; $p_{1,5;\ 2,6} < 0.01$	$p_{5,6;\ 5,7;\ 5,8} < 0.05$; $p_{6,8} > 0.05$; $p_{1,5;\ 2,6;\ 3,7;\ 4,8} < 0.01$

Also, when mice were given an oral dose of 1.5 mg/g of *P. polyphylla* rhizome extract, the AST enzyme values were similar to those of the group not injected with cyclophosphamide, and the ALT enzyme values were lower than those treated with silymarin 0.1 mg/g. The above results show that the harmful effects of cyclophosphamide in mice given an oral dose of 0.75 mg/g of *P. polyphylla* rhizome extract were reduced, and the liver was protected.

Glutathione (GSH) is the most common intracellular antioxidant of the thiol group. It plays a role in protecting cells from the harmful effects of free radicals. Additionally, it reduces endogenous oxidants and counteracts exogenous oxidative stress.[40,41] The study by Lu *et al* reported that inducing liver injury with cyclophosphamide reduced GSH levels in the liver.[42] Also, in this experimental model, the GSH content in the liver of the disease control group (4311.81 ± 164.63 nmol/g) was lower than the physiological control group (6638.83 ± 392.87 nmol/g) by 1.54 times. After the mice's CYP injections and oral administration (0.75 mg/g) of *P. polyphylla* rhizome extract, the GSH concentration in the mouse liver increased similarly to the dose of those treated with silymarin (5838.61 ± 231.68 nmol/g).

One of the main features of cyclophosphamide-induced hepatotoxicity is lipid peroxidation. Free radicals attack cell membranes leading to lipid peroxidation, and the main product is MDA. Therefore, MDA is used as a biomarker of lipid peroxidation-induced injury.[26,42] A hepatoprotective agent is considered effective when it reduces MDA content in liver tissue.[23,26] The study results showed that the mice not injected with CYP had MDA content (150.30 ± 9.92 nmol/g) 1.63 times as high as that of the mice in the group injected with CYP ($p<0.05$). After the mice orally absorbed *P. polyphylla* rhizome extract (0.75 mg/g), the MDA content was similar to that of the non-CYP (-) group. In addition, the mice orally took in a dose of 1.5 mg/g of *P. polyphylla* rhizome extract showed to have similar MDA content to the mice with cyclophosphamide-induced liver injury.

Thus, the present study provides evidence that *P. polyphylla* rhizome extract has protective effects on the liver against oxidative damage induced by CYP (+) through decreasing AST and ALT enzyme levels and increasing endogenous antioxidant GSH enzyme content in mouse liver. As a result, *P. polyphylla* rhizome extract reduced the increase in MDA content in mouse liver. Interestingly, this effect was similar to that of silymarin (0.1 mg/g of body weight).

In terms of histopathology, histological observations provided supporting evidence for biochemical analysis. The structure of liver tissue of diseased mice treated with silymarin and *P. polyphylla* rhizome extract was significantly improved. In the liver tissue of the diseased mice treated with silymarin, there were liver cells with round nuclei arranged in rows radiating from the central vein; the liver sinuses were visible and inflammatory cells were greatly reduced. As presented, the hepatoprotective activity of *P. polyphylla* rhizome extract was as effective as that of the extracts of *Ganoderma lucidium* (Curtis) P. Karst,[26] *Odontonema cuspidatum* (Nees) Kuntze,[35] *Fraxinus floribunda* Wall.,[43] this protective mechanism is known to be related to the maintenance of the antioxidant defence system in the liver along with free radical scavenging activity, which is similar to that of silymarin reported previously.[36,37]

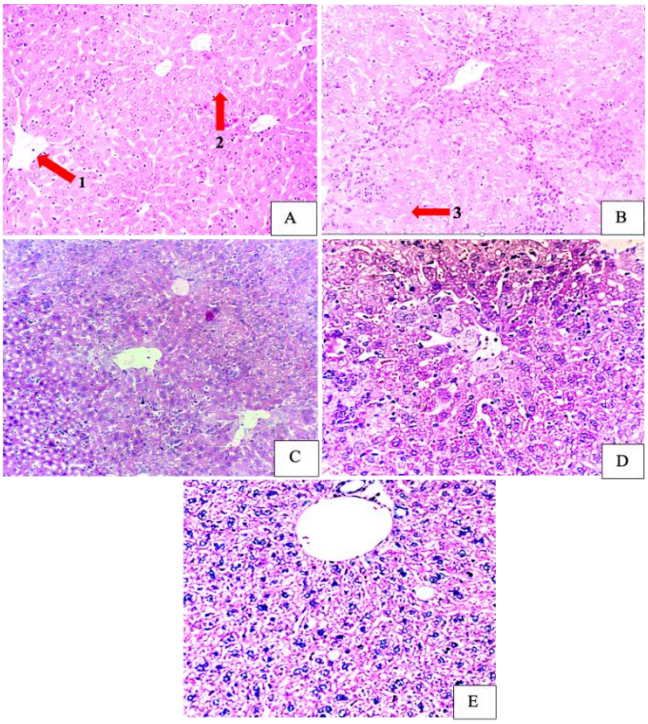

Figure 1: Photomicrograph of the liver of mice obtained from different treatment groups. (A) Group CYP (-) (drinking distilled water); (B) Pathological control (CYP (+)); (C) Lot 2 (treated with *P. polyphylla* extract, 0.75 mg/g); (D) Lot 3 (treated with *P. polyphylla* extract, 1.5 mg/g); (E) Positive control: Silymarin (0.1 mg/g); (1) Central vein; (2) Normal hepatocytes; (3) Hepatocytes ballooning. All images were observed under a 40X magnification microscope.

The results of the histopathological analysis in the experimental lot of CYP (+)

Histopathological analysis of the non-CYP (-) group showed a normal liver structure with well-rounded hepatocytes forming rows of veins, clearly visible in the hepatic sinuses (Figure 1A), while the CYP (+) group indicated structural abnormality of the liver (Figure 1B). Specifically, the pathological control lot had many necrotic and denuclearized cells. Moreover, hepatocytes with lipid droplet accumulation became ballooning and fatty, or the membranes of two adjacent cells were fused, leading to the inability to identify the hepatic sinus. In addition, many inflammatory cells were around the lobules, perivascular, and intercellular cores. Regarding the lot treated with *P. polyphylla* rhizome extract (0.75 mg/g) (Figure 1C), hepatocyte structure significantly recovered compared with the pathological control lot. Meanwhile, lot 4 (1.5 mg/g) suggested that liver tissue with necrotic cells, lipid accumulation in cells, and inflammatory cells were gradually reduced (Figure 1D). The silymarin-treated lot (Figure 1E) had hepatocytes with round nuclei, which were in vein-oriented rows, resulting in the hepatic sinuses observation. Additionally, inflammatory cells were considerably reduced and mainly present around the vascular structures. The results were similar to the normal control lot that received only distilled water.

Conclusion

This study suggested that *P. polyphylla* rhizome extract has the ability to prevent oxidation in vitro and demonstrate the hepatoprotective effect of *P. polyphylla* rhizome extract in vivo. Specifically, *P. polyphylla* rhizome extract (0.75 mg/g) was equivalent to silymarin (0.1 mg/g). Then, at a dose of 1.5 mg/g, *P. polyphylla* rhizome extract could bring the AST enzyme activity of the mouse liver to a normal value. Besides, under oxidative stress in the mouse liver, *P. polyphylla* rhizome extract had many typical effects, including reducing MDA content, increasing GSH content in liver tissue, and restoring liver tissue damage by Cyclophosphamide. This result provided evidence of liver protection of *P. polyphylla* rhizome extract.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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